Interactions between Autophagy and the Unfolded Protein Response: Implications for Inflammatory Bowel Disease

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Short title: Autophagy and UPR in IBD

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Abstract

Inflammatory Bowel Disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis, is characterised by chronic inflammation of the gastrointestinal tract. Aetiology involves a combination of genetic and environmental factors resulting in abnormal immune responses to intestinal microbiota. Genetic studies have strongly linked genes involved in autophagy to CD, and genes involved in the unfolded protein response (UPR) to IBD. The UPR is triggered in response to accumulation of misfolded proteins in the endoplasmic reticulum (ER) and autophagy plays a key role to relieve ER-stress and restore homeostasis. This review summarises the known interactions between autophagy and the UPR and discusses the impact of these converging pathways on IBD pathogenesis. With a paucity of effective long-term treatments for IBD, targeting of synergistic pathways may provide novel and more effective therapeutic options.

Keywords: IBD, autophagy, unfolded protein response, ER stress.

Introduction

Inflammatory Bowel Disease (IBD) is a group of inflammatory diseases that includes Crohn's disease (CD), ulcerative colitis (UC) and IBD unclassified (IBDU). The incidence rate for IBD is approximately 50-200 in 100,000 persons per year in Western countries [1] and following diagnosis the natural history of the condition is characterized by periods of relapse and remission, with symptoms commonly including abdominal pain, chronic diarrhoea, weight loss and lethargy [2]. CD is distinguished from UC due to the presence of submucosal or transmural inflammation and macroscopic changes that often occur in a non-contiguous pattern anywhere within the digestive tract [1]. UC is localised to the colon and inflammation is limited to the mucosa and epithelial lining of the gastrointestinal (GI) tract [2]. Patients can be diagnosed with IBDU when a conclusive distinction between CD and UC cannot be made, although this may well represent a distinct sub-type. At present there is no cure for IBD and medications such as corticosteroids, aminosalicylates, immunomodulators and biological agents are aimed at inducing and maintaining remission of disease by modifying inflammatory processes [3].

The aetiopathology of IBD is multifactorial in nature, with genetic predisposition, environmental triggers (e.g. smoking, appendicectomy, diet, pollution, antibiotics and stress) and a dysregulated mucosal immune response contributing to disease [4]. Examination of the gut microbiome has revealed that IBD is associated with microbial dysbiosis, including an expansion of facultative anaerobic bacteria of the family Enterobacteriaceae [5]. Several potentially causative agents have been identified, most notably *Escherichia coli* strains with an adherent and invasive phenotype (AIEC) are associated with ileal mucosa in CD [6]. Genome wide association studies (GWAS) have identified 240 IBD susceptibility loci to date

[7], and have confirmed association with previously recognised susceptibility genes including *Nucleotide-binding oligomerisation domain-containing protein 2* (*NOD2).* GWAS have also identified the strong association of CD with genes involved in the autophagy pathway, including *autophagy-related protein* (*ATG*)*16L1, Immunity-related GTPase family M protein* (*IRGM*) and *leucine rich repeat kinase 2* (*LRRK2*) [8]. The strong association of IBD with endoplasmic reticulum (ER) stress/Unfolded protein response (UPR) genes including *x-box-binding protein 1* (*XBP1*) [9] and genes involved in intestinal barrier function such as *MUC2* [10] and *Anterior gradient 2* (*AGR2*) [11] have been detected by gene targeted approaches. Together, these genetic studies have led to increased research exploring links between autophagy and ER stress/UPR dysregulation and IBD pathogenesis.

Autophagy

Autophagy is an intracellular process that plays an important housekeeping role by degrading excessive, damaged or aged proteins and organelles to maintain cellular homeostasis [12]. Basal autophagy is tightly regulated by the coordinated activity of autophagy-related (ATG) proteins [13] and constitutes an important survival mechanism induced in response to multiple stress conditions such as nutrient deprivation, hypoxia, DNA damage or intracellular pathogens [12]. There are three main types of autophagy in mammalian cells; macroautophagy (herein referred to as autophagy), microautophagy and chaperone-mediated autophagy [12].

When autophagy is initiated a double membrane vesicle is formed (the autophagosome) around the cargo to be degraded (**Figure 1**). The mature autophagosome then fuses with a lysosome to form an autophagolysosome, in which lysosomal enzymes degrade the inner

membrane and cargo and the resulting macromolecules are released into the cytosol for recycling (**Figure 1**).

Selective types of autophagy also exist, including autophagy of microorganisms (xenophagy) and autophagy of the ER membrane (ER-phagy), which use specific receptors and adaptor proteins to link the cargo to the autophagy machinery [14]. For example, Sequestosome 1/p62-like receptors (SLRs) target cytosolic pathogens and other cargo to initiate autophagy [15]. SLRs function by binding to the small regulatory protein ubiquitin on the surface of cargo [16–18] and subsequently associate with the autophagy machinery via a binding motif called the LC3-interacting region (LIR) [19]. Adaptor proteins, such as autophagy-linked FYVE protein (ALFY), can also bind ubiquitinated pathogens via p62 to promote association with the autophagy machinery [20]. To date, five main types of SLR have been described; sequestosome 1/p62, optineurin [18], NBR1 (Neighbor of BRCA1 gene 1) [21], NDP52 (Nuclear Domain 10 Protein 52) [17] and the NDP52-like receptor calcoco3 (Calcium-binding and coiled-coil domain-containing protein 3) [22], and specific cargo receptors are important for distinct types of selective autophagy. For example, a recent study has shown that the non-canonical cargo receptor cell-cycle progression gene 1 (CCPG1) is essential for ER-phagy [23], while another study demonstrated an integral role for optineurin in the maintenance of ER homeostasis by assisting the removal of hyper-activated UPR kinases [24].

Autophagy and CD

Autophagy affects many essential cellular processes and dysregulation of autophagy has been linked to a multitude of human diseases [25]. Autophagy plays an important role in both innate and adaptive immune signalling pathways and loss of immune regulation is a key event

leading to the chronic inflammation observed in CD [26]. Impaired autophagy responses have been observed in a range of cell types derived from CD patients including the specialized intestinal epithelial cells (IECs) Paneth cells and goblet cells, and leukocytes, such as macrophages and dendritic cells [27].

Functional studies have linked impaired autophagy to CD-associated genetic variants in *NOD2*, *ATG16L1*, *IRGM* and *LRRK2*. The single nucleotide polymorphism (SNP) in *ATG16L1* causes a single amino acid change from threonine to alanine at position 300 (*T300A*) [28], which is associated with Paneth cell and goblet cell dysfunction, and significantly impairs autophagic clearance of pathogens [29–32]. IRGM is required for the initiation of xenophagy and the clearance of intracellular organisms such as *Mycobacterium tuberculosis* [33] and dysregulation of IRGM expression compromises the control of intracellular replication of CD-associated adherent invasive *Escherichia coli* (AIEC) by autophagy [34]. LRRK2 expression is increased in colonic biopsy specimens from patients with CD [35] and functionally LRRK2 can enhance NFκB-dependent transcription, while small interfering RNA [siRNA] knockdown of LRRK2 interferes with bacterial killing [35].

NOD2 is a member of the Nod-like receptor (NLR) family of pattern recognition receptors (PRR) and recognises a component of the bacterial cell wall muramyl dipeptide (MDP) to induce innate immune responses [36]. CD-associated *NOD2* SNPs (R702W, G908R and L1007fs) affect the leucine rich repeat domain disrupting interaction with MDP and abrogating immune responses initiated by this receptor [37]. The immunoregulatory properties of NOD2 have also been linked to autophagy, and CD susceptibility is heightened when *ATG16L1* and *NOD2* variants present in combination, causing synergistic genetic epistasis [38,39]. A direct functional interaction between these proteins has been

determined; NOD2 was shown to recruit ATG16L1 to the plasma membrane to initiate autophagy at the sites of bacterial entry [40], and in a separate study IRGM was shown to regulate the formation of a complex containing NOD2 and ATG16L1 that is necessary for the induction of xenophagy [41]. The interaction of IRGM with NOD2 also stimulates phosphorylation cascades involving AMPK, ULK1 and Beclin1 that regulate autophagy initiation complexes [41]. Cells harbouring CD-associated *NOD2* variants and/or the *ATG16L1 T300A* variant exhibit a number of disrupted functions linked to autophagy including reduced production of antimicrobial peptides, enhanced pro-inflammatory responses and aberrant activation of adaptive immune responses [40,42–44].

Significantly, abnormalities in the secretory capacity of Paneth cells are observed in mice deficient for ATG16L1 [30,45,46], NOD2 [47,48], IRGM [49] and LRRK2 [50] indicating that autophagy plays an essential and specific role in Paneth cell function. Despite the significant effects on Paneth cell function, mouse strains developed for deficiency in functional ATG16L1 do not exhibit spontaneous intestinal inflammation [29–31]. In contrast, a mouse strain with targeted deletion of *ATG16L1* in IECs developed a spontaneous transmural ileitis similar to ileal CD [24]. Furthermore, targeted deletion of *ATG16L* in haematopoietic cells can enhance susceptibility to DSS-induced acute intestinal injury in mice [51] and ATG16L1 deficiency in myeloid cells in a mouse strain led to disrupted macrophage function and bacterial clearance [52]. Murine models with non-functional NOD2 do not develop spontaneous colitis [53], however a *NOD2* mutation similar to the L1007fs mutation increased susceptibility to DSS-induced colitis in mice [54]. *Irgm1*-deficient mice also exhibit abnormalities in Paneth cells, accompanied by increased susceptibility to inflammation in the colon and ileum [49]. Finally, *LRRK2* deficiency confers enhanced susceptibility to experimental colitis in mice, which was

associated with enhanced nuclear localisation of the transcription factor nuclear factor of activated T cells (NFAT1), important for regulating innate immune responses [55].

ER-stress and UPR signalling

ER stress results from accumulation of unfolded and misfolded protein in the ER, and the UPR is activated to resolve ER stress and restore homeostasis. The UPR inhibits protein synthesis, promotes protein re-folding, and induces degradation of unfolded and misfolded proteins through ER-associated protein degradation (ERAD) and autophagy (**Figure 2**). If these survival mechanisms are unsuccessful, the UPR can induce apoptosis [56]. The major regulators of the UPR are the ER-membrane resident proteins PERK (protein kinase RNA-like endoplasmic reticulum kinase), inositol-requiring transmembrane kinase endonuclease 1 (IRE1) and activated transcription factor (ATF)6. When inactive these proteins are bound to binding immunoglobulin protein (BiP), also known as glucose regulated protein 78 (GRP78) [57]. During ER stress, BiP binds to misfolded proteins in the ER and dissociates from the ER-membrane resident proteins to allow their transition to an active state [57] (**Figure 2**).

When active, PERK phosphorylates elongation initiation factor 2α (EIF2α), to inhibit general protein synthesis [58] and specifically up-regulates ATF4 [59]. ATF4 in turn transcriptionally up-regulates several other UPR genes including CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) [60,61] (**Figure 2**). CHOP is also a transcription factor that regulates several other UPR genes, and under conditions of prolonged ER stress can promote apoptosis [60,61].

IRE1 exists in two forms: IRE1α that is ubiquitously expressed and IRE1β that is only expressed in the GI tract and lung epithelial cells [62]. During ER stress, IRE1 is activated through

dimerization and auto-phosphorylation [63,64]. The IRE1α RNase domain is essential for creating transcriptionally activate *XBP1* messenger RNA (mRNA) via splicing, which acts as a transactivator of UPR genes [65–68] (**Figure 2**) . IRE1 endoribonuclease activity also facilitates degradation of specific mRNA in a process known as RIDD (regulated IRE1-dependent decay) [69].

ATF6 translocates to the Golgi apparatus once released from its complex with BiP [70]. This allows cleavage by site 1 and site 2 proteases (S1P and S2P), which releases the transcriptionally active cytoplasmic domain of ATF6 (ATF6-N) that induces UPR-associated genes [71–73] (**Figure 2**). Among the ATF6 upregulated genes are *CHOP* and *XBP1* [74].

ER-stress, UPR and intestinal inflammation

Genetic studies have identified several ER-stress/UPR genes associated with IBD [75]. Moreover, ER-stress levels are increased in ileal and colonic biopsies from CD patients, with higher than normal levels of BiP, chaperone protein Gp96, and spliced *XBP1* observed [9,76–78] (**Table 1**). Several studies have focused on IRE1-XBP1 signalling in murine models. In mice with targeted deletion of XBP1 in intestinal epithelial cells (IECs) ($XBP1^{\Delta IEC}$ mice), spontaneous inflammation of the small intestine, increased susceptibility to DSS-induced colitis and elevated levels of ER stress were observed [9] (**Table 1**). Furthermore, in $XBP1^{\Delta IEC}$ mice increased levels of apoptosis were observed along with reduced goblet cell and Paneth cell numbers, leading to decreased production of host defence peptides and higher susceptibility to *Listeria monocytogenes* infection [9] (**Table 1**). *XBP1* has also been shown to suppress experimental colitis-associated cancer [79], and is essential for efficient TLR-mediated pro-

inflammatory responses to infection in macrophages [80]. These studies support *XBP1* as a key component of the protective function of IECs and macrophages.

Although the UPR acts to maintain ER-homeostasis, hyper-activation of certain UPR components can create a pro-inflammatory state. In *XBP1*$^{\Delta IEC}$ mice increased activation of IRE1α, causes hyper activation of NFκB, and spontaneous inflammation [45] (**Table 1**). IRE1β knock-out mice have enhanced sensitivity to DSS-induced colitis [81] and exhibit goblet cell abnormalities with exaggerated MUC2 accumulation (**Table 1**). In contrast, IRE1α knock-out mice have normal goblet cells [82]. In murine Paneth cells, IRE1α and IRE1β have distinct roles with hyper activation of IRE1α driving CD-like ileitis, and IRE1β having a protective role [24].

Association of aberrant PERK-EIF2α and ATF6 pathways with intestinal inflammation have also been identified. A mouse model expressing non-phosphorylatable EIF2α in IECs resulted in functional abnormalities in Paneth cells and increased susceptibility to *Salmonella* infection and DSS-induced colitis [83] (**Table 1**). ATF6α deficient mice exhibit increased ER stress as indicated by elevated levels of BiP, ATF4, CHOP and spliced *XBP1,* which result in enhanced sensitivity to DSS-induced colitis [84] (**Table 1**). Additionally, hypomorphoic mutation in *membrane-bound transcription factor peptidase S1P-encoding gene* (*Mbtps1*), which encodes the S1P responsible for cleavage of ATF6, causes enhanced susceptibility to DSS-induced colitis [85]. Although there is less evidence to support a role for PERK-EIF2α and ATF6 pathways in IBD pathogenesis, their importance for ER stress responses in the intestinal epithelium is clear.

ER-stress and intestinal barrier function

In the intestinal epithelium, cells that naturally secrete large amounts of protein, such as Paneth cells and goblet cells, are more susceptible to ER-stress and therefore rely heavily on the UPR to maintain homeostasis. MUC2 is the major component of mucin that is produced in goblet cells and secreted into the intestinal lumen. *Winnie* mice are characterised by a missense mutation in *MUC2,* which causes abnormalities in goblet cells, leading to aberrant mucous production and spontaneous colitis, and association with *MUC2* variants has been identified in IBD patients [86]. *Winnie* mice also exhibit severe ER stress in goblet cells [10], which causes up to four-fold increase in activated dendritic cells in the colonic lamina propria, and aberrant adaptive immune responses associated with interleukin (IL)-23/Th17 [87]. Goblet cell abnormalities are also apparent in mice deficient in UPR transcription factor OASIS, which causes increased ER stress and susceptibility to DSS-induced colitis [88,89]. AGR2 is an ER resident protein highly expressed in goblet and Paneth cells and regulates the formation of disulphide bonds in mature proteins. *AGR2-/-* mice exhibit a decreased number of goblet cells and MUC2 production, Paneth cell abnormalities, elevated ER-stress and spontaneous colitis [90]. Notably, AGR2 is decreased in patients with CD and UC [11]. These studies highlight the key role of intestinal secretory cells and breakdown of intestinal barrier function in IBD pathogenesis.

Functional intersection between autophagy and the UPR

The UPR and autophagy are intimately linked processes. In a range of Intestinal epithelial cells, chemical ER-stress inducers activate autophagy, modulated by enhanced expression of CHOP and stimulation of the IRE1α pathway [91]. In endothelial cells, IRE1α-dependent splicing of *XBP1* mRNA activated autophagy via up-regulation of *Beclin-1,* which is a major regulator of

the autophagy pathway [92] (**Figure 3**). Contrary to expectation, *XBP1* deletion in a familial amyotrophic lateral sclerosis mouse model increased autophagy, which enhanced clearance of accumulated toxic superoxide dismutase-1 (SOD1) aggregates [93]. It was suggested that in this scenario, autophagy is induced in a compensatory manner due to attenuated UPR.

The UPR and autophagy also intersect at the PERK-EIF2α-ATF4 pathway [94–99]. In an *in vitro* model of osteosarcoma, PERK induced autophagy via mechanistic target of rapamycin (mTORC1) inhibition to promote survival in response to ER stress-conferred chemoresistance to apoptosis [95] (**Figure 3**). Additionally, PERK modulates autophagy via AMPK-dependent inhibition of mTORC1 in response to extracellular matrix (ECM) detachment in mammary epithelial cells (MECs) [94]. One of the main functional outcomes of PERK signalling is reduced protein synthesis. Inhibition of mTORC1 helps to promote this effect as mTORC1 controls synthesis of ~15-20% of protein within the cell [100]. Thus, via modulation of mTORC1, PERK signalling achieves dual outcomes; inhibition of protein synthesis and induction of autophagy to degrade misfolded proteins.

During amino acid deprivation, ATF4 and CHOP can bind specific C/EBP-ATF Response Elements (CAREs), also known as Amino Acid Response Elements (AAREs) and CHOP-Response Elements (CHOP-REs) to induce transcription of a wide range of autophagy genes [101] (**Figure 3**). In other studies, hypoxia or ECM detachment induced PERK-dependent autophagy due to autophagy gene up-regulation via ATF4 and CHOP [102–104]. This up-regulation of autophagy gene transcription by the UPR was shown to replenish autophagy proteins to promote survival during cellular stress [103].

ATF6 has also been implicated mechanistically in autophagy regulation. In response to cellular stress, interferon (IFN)-γ activates the Ask1 (Apoptosis signal-regulating kinase 1)/MAPK (Mitogen-activated protein kinase) pathway, which phosphorylates ATF6 to allow its proteolytic activation [105]. ATF6 interaction with C/EBP-β is essential for IFN-γ-induced up-regulation of *DAPK1 (death-associated protein kinase 1)*, which can subsequently stimulate autophagy [106] (**Figure 3**). Mice lacking either ATF6 or Ask1 are highly susceptible to bacterial infection due to defective autophagy [105,106]. Furthermore, ATF6 recruitment of DAPK1 in response to ER stress enhanced xenophagy in human colonic biopsies and epithelial cells, which was attenuated in cells harbouring the *ATG16L1 T300A* SNP [107]. Additionally, activated ATF6 was shown to stimulate Akt (protein kinase B), which resulted in the inhibition of mTORC1 [108,109] (**Figure 3**).

In a recent study in MCF-7 human breast cancer cells, ER stress induced by the chemo-preventative agent ursolic acid (UA) was associated with autophagy activation [99]. UA induced autophagy via MAPK1/3 signalling and subsequent promotion of PERK signalling, resulting in the inhibition of apoptosis. Furthermore, a study in human ovarian cancer cells showed interdependent activation of autophagy and the PERK-EIF2α UPR pathway when treated with metformin, which causes energy starvation [98]. In these scenarios an unconventional relationship between autophagy and ER stress was uncovered, which remains to be mechanistically solved. Nonetheless, under these circumstances the interaction of the UPR and autophagy pathways has pro-survival outcomes.

Convergence of autophagy, ER-stress and CD

In an attempt to relieve ER-stress the UPR can induce autophagy to degrade misfolded proteins, protein aggregates and damaged organelles [91,110–113]. Autophagy activity is increased in highly secretory Paneth cells [45] to counterbalance high levels of ER-stress [112], thus ER-stress is a significant risk in these cells when the UPR or autophagy is not functional. Consistent with this, in Paneth cells of CD patients harbouring *ATG16L1 T300A* risk alleles, BiP and pEIF2α are highly expressed [46] (**Table 1**). Significantly, *ATG16L1;XBP1*$^{\Delta IEC}$ mice develop similar phenotypic ileitis to ATG16L1$^{\Delta IEC}$ mice, but earlier in life due to increased ER stress [24,45].

ERAD can regulate the degradation of IRE1α to prevent accumulation of toxic IRE1α aggregates, however persistent ER-stress will inhibit ERAD degradation of IRE1α [24]. When this occurs, autophagy plays an important role in the clearance of supramolecular clusters of IRE1α (**Figure 3**). In *ATG16L1*$^{\Delta IEC}$ mice, development of spontaneous CD-like ileitis is associated with defective autophagy resulting in toxic accumulation of IRE1α in Paneth cells [24] (**Table 1**). Furthermore, the selective autophagy receptor optineurin interacts with IRE1α, and optineurin deficiency amplified the accumulation of IRE1α [24]. In humans homozygous for *ATG16L1 T300A*, a similar accumulation of IRE1α was observed in intestinal epithelial crypts [24] (**Table 1**). This has led to suggestion that the *ATG16L1 T300A* SNP may define a specific subtype of patients with CD, characterised by Paneth cell ER-stress [46]. This synergistic and compensatory relationship between the UPR and autophagy is affirmed by the presence of CD-associated SNPs in *ATG16L1* and *XBP1*.

A recent study has demonstrated a direct link between NOD1/2 and the IRE1α pathway in the context of ER-stress-induced inflammation [114]. When active, IRE1α stimulates the c-Jun N-terminal kinase (JNK) pathway and recruits TRAF2 (TNF receptor-associated factor 2) to the

ER membrane to trigger NFκB signalling [115,116] and autophagy induction [112,117,118] (**Figure 3**). In mouse and human cells, ER-stress induced by chemicals or infection with *Brucella abortus* and *Chlamydia muridarum* increased inflammation and IL-6 production [114]. This response was dependent on NOD1/2 and receptor-interacting serine/threonine-protein kinase 2 (RIPK2), but also on IRE1α kinase activity and TRAF2-induced NFκB signalling [114]. This suggests there is a functional intersection between the IRE1α pathway and NOD1/2 signalling, which is facilitated by TRAF2 (**Figure 3**).

Interestingly, an additional study has shown that ER-stress responses can be modulated by another innate immune sensor called stimulator of interferon genes (STING) in response to cyclic-di-AMP (c-di-AMP), a vita-PAMP (pathogen associated molecular pattern) present in live Gram-positive bacteria [119]. This process induces autophagy via inhibition of the major autophagy suppressor mTORC1 and localisation of STING to autophagosomes.

Pharmacological induction of autophagy and the UPR

A recent review estimated IBD treatment costs of £720 million ($940m) per year in the United Kingdom alone [120], with roughly a quarter of these costs directly attributed to drug treatments [121]. The efficacy of these drugs continues to come under scrutiny as response to treatment often diminishes over time, with a review of worldwide cohorts estimating that between 10–35% of CD patients required surgery within a year of diagnosis and up to 61% by 10 years [122]. In order to improve the efficacy of IBD treatment, optimization of existing clinical therapies and the development of novel therapeutics is required.

The convergence between autophagy and UPR pathways provides new opportunity for the treatment of IBD and the modulation of the UPR in combination with autophagy inducers is a

promising therapeutic strategy. There is evidence that inducing autophagy can have therapeutic benefits for the treatment of IBD [26] with several studies investigating the utility of autophagy inducers as adjuvant therapies. Rapamycin analogues, sirolimus and everolimus, inhibit mTORC1 to induce autophagy and are already approved for clinical use for post-transplantation (e.g. liver and renal) management. In IL-10-deficient mice, everolimus treatment alleviated spontaneous colitis and reduced CD4+ T cells and IFN-γ [123]. In a case study sirolimus improved symptoms and intestinal healing in a patient with severe refractory CD [124]. In another case study, symptoms were controlled for 18 months with everolimus treatment in a refractory UC patient [125]. Moreover, in a study of refractory paediatric IBD, sirolimus induced clinical remission in 45% of UC patients and 100% of CD patients; albeit the sample size was small [126]. Significantly, everolimus had comparable safety and tolerability as azathioprine when used to maintain steroid-induced remission in a cohort of adult CD patients [127]. As these mTORC1 inhibitors are already approved for clinical use, they have been investigated the most extensively, however there are a plethora of novel autophagy modulators that are currently being developed, characterised and patented for therapeutic use in a range of diseases including IBD [128,129].

Recent progress has also been made to identify specific chemical inducers of the UPR. A screen of 1,200 FDA-approved compounds carried out in *C.elegans* identified eight compounds that induced UPR responses, four of which specifically increased mitochondrial UPR [130]. The identified drugs included antirheumatic agents, antianginal calcium channel blockers; androgen receptor inhibitors used for cancer therapy and tetracycline antibiotics.

A well-characterised modulator of the UPR, tauroursodeoxycholic acid (TUDCA), that promotes protein refolding to reduce ER-stress, was shown to ameliorate DSS-induced colitis

in mice by decreasing ER-stress in IECs [84]. Furthermore, a selective inhibitor of eIF2α dephosphorylation protects cells from ER-stress and ameliorates murine experimental colitis [131,132]. Supplementation with glutamine has also been suggested for the improvement of IBD treatment, as this amino acid was shown to dampen experimental colitis in rats by inhibiting ER-stress in colonic epithelial cells [133].

Drugs used to treat metabolic disorders have also been investigated for UPR inducing properties. The biguanides metformin and phenformin have been implicated in induction of the UPR and resolution of ER-stress via activation of AMPK, which subsequently stimulated IRE1α and PERK pathways [98,134,135]. Inhibitors of dipeptidyl peptidase IV (DPP4), including gemigliptin, also prevented ER-stress-mediated apoptosis by promoting IRE1α and PERK pathways [136]. Furthermore, agonists of the glucagon-like peptide-1 receptor, such as exenatide, relieved ER stress via up-regulation of *ATF4* expression [137]. Exogenous chemical chaperones have also been explored as a method to relieve ER stress by mimicking ER chaperones to promote protein transport and re-folding capacity [138].

Although several studies have demonstrated beneficial effects of enhancing UPR function for intestinal homeostasis, future investigations should proceed with caution. For example, hyper-activation of the UPR kinase IRE1α can exacerbate intestinal inflammation, as seen in patients with ATG16L1 and NOD2 mutations, therefore, in certain circumstances pharmacological inhibition of UPR receptors would be a more effective strategy [24,45,114]

Of particular interest, the selective autophagy cargo receptor optineurin forms a critical link between ER-stress resolution and autophagy due to its role in the degradation of IRE1α aggregates [24], and another recently identified autophagy cargo receptor that is integral for resolution of ER-stress, CCPG1, mediates ER-phagy to remove damaged ER membranes [23]. Understanding the biology and functions of adaptors such as optineurin and CCPG1 may identify novel druggable targets and expedite development of the next generation of therapeutics aimed at modulation of the UPR in combination with autophagy.

Discussion

The complexity of IBD is evident from the large number of risk loci identified by genetic studies, and the diverse health profile of patients that are affected. Mouse models of IBD cannot emulate the human disease, however they are useful tools to explore how specific gene mutations influence inflammation. Interestingly, as highlighted in (**Table 1)** the majority of mouse models mimicking IBD-associated genetic risk do not develop spontaneous inflammation, but rather they are sensitised to DSS-induced colitis, which acts by damaging the epithelium and increasing intestinal permeability. The intestinal epithelium has important immunoregulatory functions and controls the equilibrium between tolerance and immunity to non-self-antigens [139]. As such breakdown of intestinal epithelial barrier function and concomitant interaction with environmental factors in the lumen is a trigger for inflammation. The intestinal lumen comprises a multitude of potential triggers including the microbiota, dietary antigens, and luminal antigens. Additional triggers may be host-derived factors that are released into the lumen as the intestinal epithelial barrier breaks down. These so-called Damage-Associated Molecular Patterns (DAMPS) include intracellular proteins, such as high-mobility group box 1 (HMGB1), heat-shock proteins and components derived from

the extracellular matrix. Examples of non-protein DAMPs include genomic DNA, mitochondrial DNA, RNA, uric acid and ATP [140,141]. Not surprisingly, there is considerable interest in developing novel therapeutic strategies aimed at re-establishing intestinal barrier function [142] and modulation of DAMPs for the treatment of IBD [140].

Dysbiosis of the gut microbiome is strongly implicated in the pathogenesis of CD [143], and it has been suggested that microbial dysbiosis may be an environmental trigger. A recent study by Tschurtschenthaler and colleagues [24] addressed this question. Although microbial dysbiosis was present in the ileum of *Atg16l1*;*Xbp1*$^{\Delta IEC}$ mice, such structural alteration of the microbiota did not trigger ileitis but, rather, aggravated DSS-induced colitis [24]. In order to understand the role of the environment in disease, determining the relative contribution of genetics and a detailed characterization of environmental triggers is required.

Greater understanding of the genetic factors that underlie CD pathogenesis are leading to improvements in treatment. Development of personalised therapies may be achieved via genotyping for key SNPs in genes involved in both the autophagy and UPR pathways. IBD drugs already established in the clinic have been shown to exert their effects, at least in-part, through the modulation of autophagy [26] or the UPR, and establishing patient genotypes may help predict response. For example, recent studies have identified an association between *ATG16L1 T300A* SNP and an enhanced therapeutic effect of thiopurines [144] and anti-TNF-α therapy [145]. Interestingly, the immunoregulatory effects of these drugs were associated with autophagy stimulation [144,146,147] and the *T300A* genotype has been associated with a subset of patients that exhibit deficiencies in both the UPR and autophagy [46]. Furthermore, CD patients harbouring *NOD2* mutations associate with better clinical outcomes in response to thiopurines, whereas CD patients with wild-type *NOD2* respond

better to steroids and anti-TNF therapy [148]. Due to the genetic complexity of IBD and epistasis between genes, it is imperative that multiple genes are analysed for the purpose of patient stratification. For example, a recent study identified a 32-gene transcriptomic signature in lymphoblastoid cells that was able to predict lack of response to thiopurines, with aberrant cell cycle control, DNA mismatched repair and RAC1-dependent mechanisms implicated in thiopurine resistance [149]. Furthermore, it is increasingly clear that epigenetic, microRNA and immune cell signatures among others will have a significant role to play in predicting disease susceptibility and response to therapy [150–152].

With regards to the intestinal microbiota, a recent study has characterised microbial signatures for the diagnosis of IBD that were highly sensitive and could differentiate CD patients from healthy controls and UC patients. This study highlights the potential for using the intestinal microbiota as a micro-biomarker [153]. Importantly, as many drugs need to be metabolised and de-toxified by the gut microbiota, this approach could also have application in predicting response to therapy. Given that dysregulation of autophagy and ER-stress can affect the intestinal microbial environment, analysis of microbial signatures may help to determine if a patient would benefit from drugs that modulate the autophagy or UPR pathways.

To conclude, the ER-stress/UPR and autophagy pathways play a vital role in the maintenance of intestinal homeostasis and breakdown of these converging pathways has been implicated in persistent intestinal infections, chronic inflammation and dysregulated immune responses observed in IBD. Therefore, strategies aimed at modulating these pathways simultaneously may prove to be an effective therapeutic option.

Funding

This work was supported by a Crohn's in Childhood Research Association (CICRA) PhD studentship to KMH. PH is supported by a NHS Research Scotland Career Researcher Fellowship.

Figure Legends

Figure 1: Autophagy pathway and autophagosome biogenesis

During the initial stages of autophagy, the isolation membrane forms a double membrane vesicle (the autophagosome) around the cargo to be degraded. ULK complex (ULK1-ULK2-ATG13-FIP200-ATG101) and Beclin 1 (Vps34-Vps150-Beclin1) complex, through interaction with ATG14, recruit autophagy proteins and complexes to the autophagosome membrane. ATG12 is conjugated to ATG5 and forms a complex with ATG16L1 (ATG16L1 complex). The ATG16L1 complex is proposed to specify the site of LC3 lipidation for autophagosome formation. LC3 is conjugated to PE to form lipidated LC3-II and is associated with the autophagosome outer membrane. Upon autophagosome closure, LC3 localises to the inner membrane and other autophagy proteins and complexes dissociate for recycling. The mature autophagosome then fuses with a lysosome to form an autophagolysosome, in which cargo are degraded by lysosomal enzymes and subunits are recycled.

Figure 2: The unfolded protein response

BiP chaperone protein binds unfolded/misfolded proteins in the ER and dissociates from transmembrane receptors upon accumulation of the toxic proteins. The transmembrane receptors PERK, IRE1α and ATF6 become activated. PERK phosphorylates EIF2α, which downregulates global translation but specifically upregulates ATF4 and CHOP that upregulate UPR-associated genes. IRE1α splices XBP1 to its active form and ATF6 is cleaved by S1P and S2P to active ATF6-N, which both translocate to the nucleus to upregulate UPR-associated genes. The main function of these UPR-associated genes is to increase protein refolding,

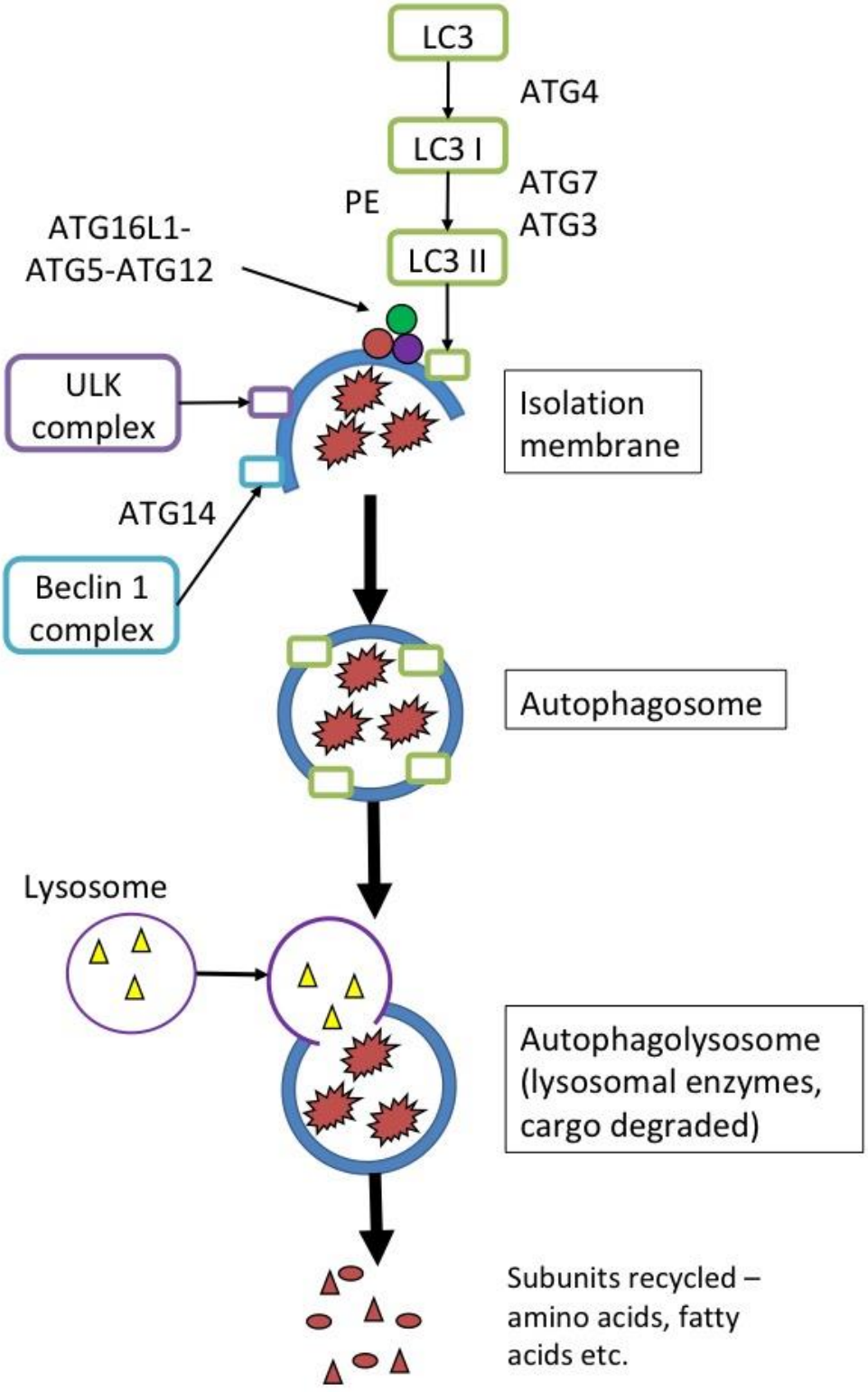
inhibit synthesis of new protein and degrade unfolded/misfolded proteins through autophagy and ERAD.

Figure 3: Intersection between autophagy and the unfolded protein response

ER stress activates transmembrane receptors PERK, IRE1α and ATF6. PERK phosphorylates EIF2α, which specifically upregulates ATF4 and CHOP that bind AAREs and CHOP-Res to upregulate autophagy genes. PERK also induces autophagy via mTORC1 inhibition. IRE1α splices XBP1 to its active form, which up-regulates *Beclin-1*. IRE1α endonuclease activity activates the JNK pathway, which induces autophagy via TRAF2, NOD2 and NFκB. Enhanced autophagy degrades accumulated IRE1α clusters. Active ATF6-N induces autophagy via mTORC1 inhibition and binds C/EBP-β to up-regulate *DAPK1*.

Table 1: Murine models of intestinal inflammation

Links between autophagy, ER-stress/UPR and experimental colitis/intestinal inflammation and IBD.



473

474 Figure 1

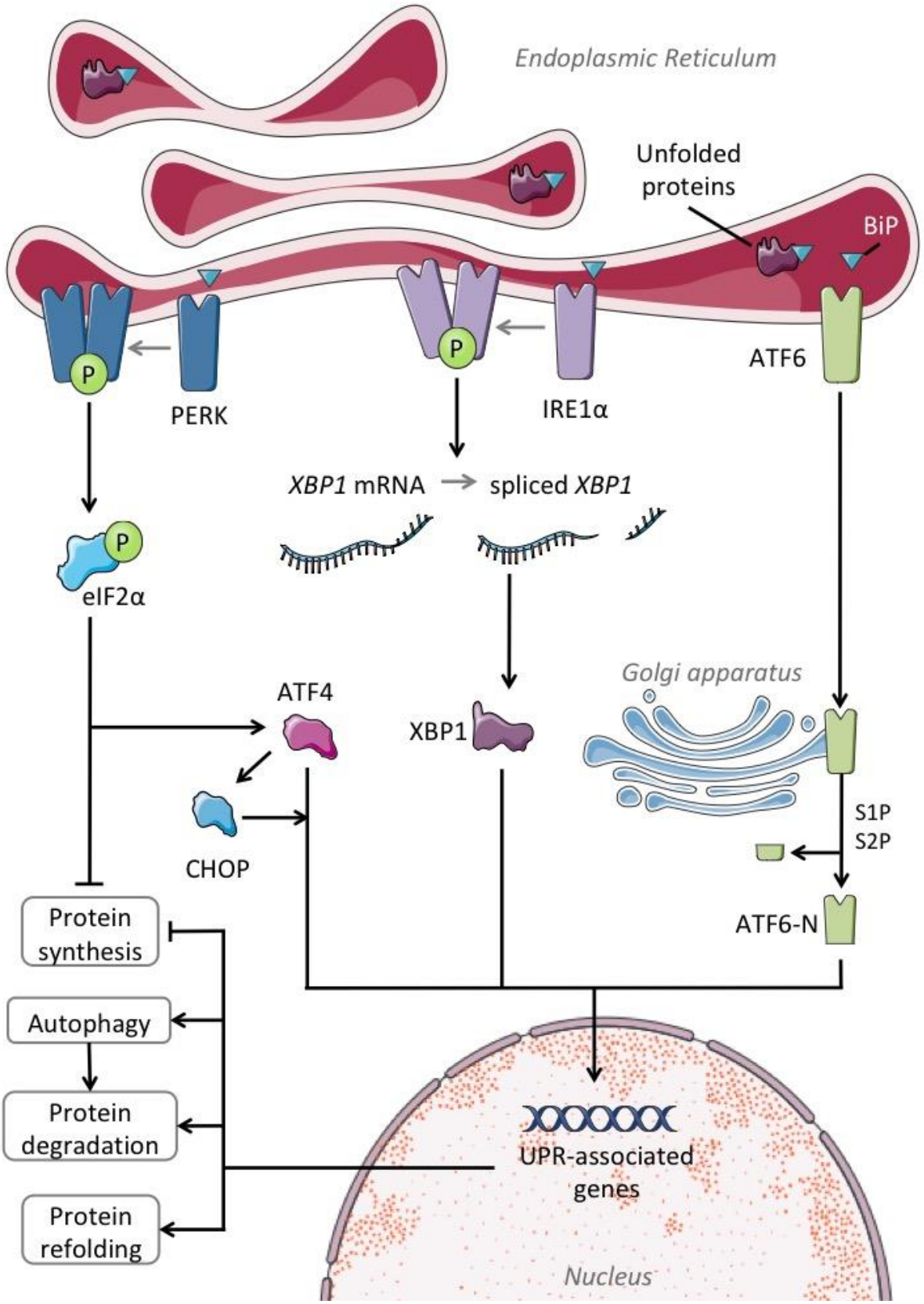

Endoplasmic Reticulum
Unfolded proteins
BiP
ATF6
P
PERK
P
IRE1α
XBP1 mRNA
spliced XBP1
P
eIF2α
ATF4
XBP1
Golgi apparatus
S1P
S2P
CHOP
ATF6-N
Protein synthesis
Autophagy
Protein degradation
Protein refolding
UPR-associated genes
Nucleus

Figure 2

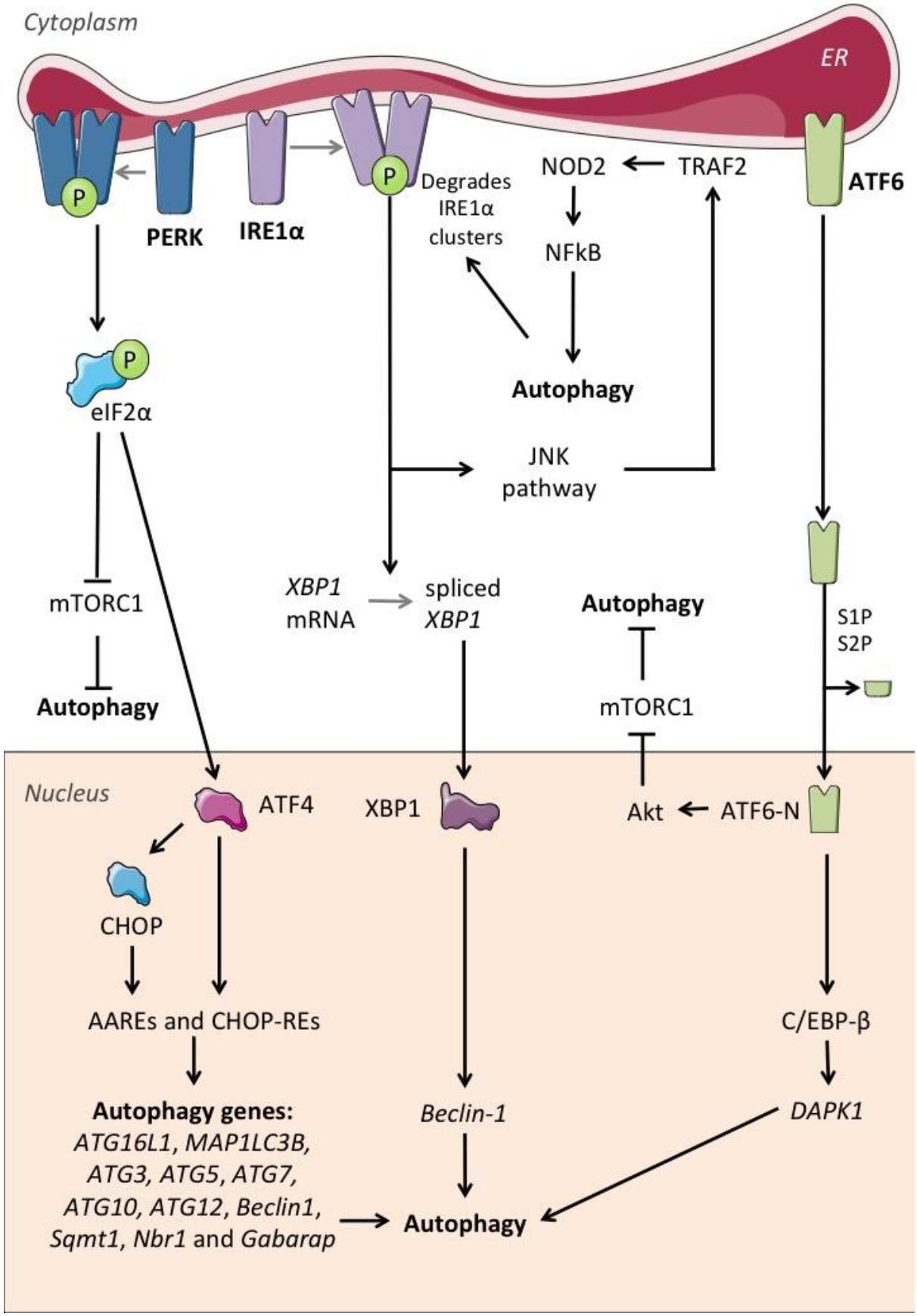

Cytoplasm
ER
P
PERK
IRE1α
P
Degrades
IRE1α
clusters
NOD2
TRAF2
ATF6
NFkB
Autophagy
P
eIF2α
JNK
pathway
mTORC1
Autophagy
XBP1
mRNA
spliced
XBP1
Autophagy
mTORC1
S1P
S2P
Nucleus
ATF4
XBP1
Akt
ATF6-N
CHOP
AAREs and CHOP-REs
C/EBP-β
Autophagy genes:
ATG16L1, MAP1LC3B,
ATG3, ATG5, ATG7,
ATG10, ATG12, Beclin1,
Sqmt1, Nbr1 and Gabarap
Beclin-1
DAPK1
Autophagy

Figure 3

Autophagy/ UPR pathway	Murine models of intestinal inflammation	IBD patients
ATG16L1	• ATG16L1 deficiency caused enhanced susceptibility to experimental colitis, Paneth cell and Goblet cell dysfunction, disrupted macrophage function and significantly impairs xenophagy [29-32, 51, 52] • ATG16L1 deletion in IECs induced spontaneous transmural ileitis [24]	*ATG16L1 T300A* CD-associated SNP [28]
NOD2	NOD2 mutation causes enhanced susceptibility to DSS-induced colitis [54] and causes Paneth cell dysfunction [47, 48]	*NOD2* CD-associated SNPs (R702W, G908R and L1007fs) [37]
IRGM	*Irgm1* deficiency causes abnormalities in Paneth cells and increased susceptibility to inflammation in the colon and ileum [49]	*IRGM* CD-associated SNP [8]
LRRK4	LRRK2 deficiency confers enhanced susceptibility to experimental colitis in mice [55] and Paneth cell abnormalities [50]	*LRRK4* CD-associated SNP [8]
IRE1α-XBP1	• *XBP1* deletion causes spontaneous intestinal inflammtaion, abnormal Paneth and goblet cell function and increased infection [9] • *XBP1* deletion causes overactivation of IRE1α and NFκB [45] • *ATG16L1* deletion causes accumulation of IRE1α in Paneth cells resulting in CD-like ileitis [24]	• *XBP1* CD-associated SNP [9] • Increased levels of spliced *XBP1,* BiP and Gp96 in CD [9, 76-78] • *T300A* SNP causes accumulation of IRE1α in intestinal crypts [24]
IRE1β	IRE1β deletion causes enhanced sensitivity to DSS-colitis [81], goblet cell abnormalities and MUC2 accumulation [24]	
PERK-EIF2α	Non-phosphorylatable EIF2α caused Paneth cell abnormalities, enhanced DSS-colitis susceptibility and increased *Salmonella* infection [83]	Increased p-EIF2α and BiP in CD patients with *T300A* SNP [46]
ATF6	• ATF6 deletion enhanced DSS-colitis susceptibility [84] • Mutation in *Mbtps1* (encodes S1P) causes enhanced DSS-colitis susceptibility [85]	
AGR2	AGR2 deletion causes decreased Goblet cells and MUC2 production, Paneth cell abnormalities, elevated ER-stress and spontaneous colitis [90]	• *AGR2* CD-associated SNP [11] • AGR2 decreased in IBD [11]

480 Table 1

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